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54. Title of the invention:

Method of producing protein having factor VII activity

57. Abstract:

Problem: Novel method of producing protein having factor VIIa activity.

Solution: Method characterized in that mammalian cells are cultured into which DNA coding for protein having factor VIIa activity after activation has been inserted, and protein is obtained from this culture fluid and activated.

PATENT CLAIMS:

1. A method of producing protein having biological activity for blood coagulation mediated by factor VIIa comprising growing in an appropriate culture medium mammalian host cells containing a DNA construct containing a nucleotide sequence coding for a protein having the same or essentially the same biological activity for blood coagulation as factor VIIa having the following amino acid sequence:

[see extra sheet]

isolating the protein product encoded by said DNA construct and produced by said mammalian host cells, and activating said protein product and generating protein which has the same or substantially the same biological activity for blood coagulation as factor VIIa.

2. A method according to claim 1, including amplification of said DNA construct by cotransfection of said host cells with a gene coding for dihydrofolate reductase, wherein said appropriate medium contains methotrexate.

3. A method according to claim 1, wherein said protein product is activated by reacting it with a proteolytic enzyme selected from the group consisting of factor XIIa, factor IXa, kallikrein, factor Xa, and thrombin.

1	5	10	15											
Glu	Cys	Lys	Glu	Glu	Gln	Cys	Ser	Phe	Glu	Glu	Ala	Arg	Glu	Ile
20			25											30
Phe	Lys	Asp	Ala	Glu	Arg	Thr	Lys	Leu	Phe	Trp	Ile	Ser	Tyr	Ser
35				40										45
Asp	Gly	Asp	Gln	Cys	Ala	Ser	Ser	Pro	Cys	Gln	Asn	Gly	Gly	Ser
50				55										60
Cys	Lys	Asp	Gln	Leu	Gln	Ser	Tyr	Ile	Cys	Phe	Cys	Leu	Pro	Ala
65				70										75
Phe	Glu	Gly	Arg	Asn	Cys	Glu	Thr	His	Lys	Asp	Asp	Gln	Leu	Ile
80				85										90
Cys	Val	Asn	Glu	Asn	Gly	Gly	Cys	Glu	Gln	Tyr	Cys	Ser	Asp	His
95				100										105
Thr	Gly	Thr	Lys	Arg	Ser	Cys	Arg	Cys	His	Glu	Gly	Tyr	Ser	Leu
110				115										120
Leu	Ala	Asp	Gly	Val	Ser	Cys	Thr	Pro	Thr	Val	Glu	Tyr	Pro	Cys
125				130										135
Gly	Lys	Ile	Pro	Ile	Leu	Glu	Lys	Arg	Asn	Ala	Ser	Lys	Pro	Gln
140				145										150
Gly	Arg	Ile	Val	Gly	Gly	Lys	Val	Cys	Pro	Lys	Gly	Glu	Cys	Pro
155				160										165
Trp	Gln	Val	Leu	Leu	Leu	Val	Asn	Gly	Ala	Gln	Leu	Cys	Gly	Gly
170				175										180
Thr	Leu	Ile	Asn	Thr	Ile	Trp	Val	Val	Ser	Ala	Ala	His	Cys	Phe
185				190										195
Asp	Lys	Ile	Lys	Asn	Trp	Arg	Asn	Leu	Ile	Ala	Val	Leu	Gly	Glu
200				205										210
His	Asp	Leu	Ser	Glu	His	Asp	Gly	Asp	Glu	Gln	Ser	Arg	Arg	Val
215				220										225
Ala	Gln	Val	Ile	Ile	Pro	Ser	Thr	Tyr	Val	Pro	Gly	Thr	Thr	Asn
230				235										240
His	Asp	Ile	Ala	Leu	Leu	Arg	Leu	His	Gln	Pro	Val	Val	Leu	Thr
245				250										255
Asp	His	Val	Val	Pro	Leu	Cys	Leu	Pro	Glu	Arg	Thr	Phe	Ser	Glu
260				265										270
Arg	Thr	Leu	Ala	Phe	Val	Arg	Phe	Ser	Leu	Val	Ser	Gly	Trp	Gly
275				280										285
Gln	Leu	Leu	Asp	Arg	Gly	Ala	Thr	Ala	Leu	Glu	Leu	Met	Val	Leu
290				295										300
Asn	Val	Pro	Arg	Leu	Met	Thr	Gln	Asp	Cys	Leu	Gln	Gln	Ser	Arg
305				310										315
Lys	Val	Gly	Asp	Ser	Pro	Asn	Ile	Thr	Glu	Tyr	Met	Phe	Cys	Ala
320				325										330
Gly	Tyr	Ser	Asp	Gly	Ser	Lys	Asp	Ser	Cys	Lys	Gly	Asp	Ser	Gly
335				340										345
Gly	Pro	His	Ala	Thr	His	Tyr	Arg	Gly	Thr	Trp	Tyr	Leu	Thr	Gly
350				355										360
Ile	Val	Ser	Trp	Gly	Gln	Gly	Cys	Ala	Thr	Val	Gly	His	Phe	Gly
365				370										375
Val	Tyr	Thr	Arg	Val	Ser	Gln	Tyr	Ile	Glu	Trp	Leu	Gln	Lys	Leu
380				385										390
Met	Arg	Ser	Glu	Pro	Arg	Pro	Gly	Val	Leu	Leu	Arg	Ala	Pro	Phe
395				400										405

Pro

Figure 5 illustrates the Factor VII cDNA sequence of λ VII 2463.
Figure 6 illustrates the Factor VII cDNA sequence of λ VII 2463.
Figure 7 illustrates the Factor VII cDNA sequence of λ VII 2463.